NTP Archives: The Ex-Files

Not far from the NIEHS campus in Research Triangle Park, North Carolina, is a little-known national treasure. The National Toxicology Program (NTP) archives is the repository for supporting documentation from 30 years' worth of toxicological research. The NTP is a federal entity that conducts congressionally mandated research on substances to determine their potential for causing cancer and other health effects in humans. When the NTP archives opened in 1984, it inherited many specimens from the National Cancer Institute, some of which date back to the late 1960s. There are currently data on hand from over 1,000 different studies. These archives provide today's scientists with a treasure trove of historical scientific information that has surprisingly current applications.

What's in the Archives . . .

After animals are sacrificed for a research study, they are necropsied. The sections of tissue that are to be examined are encased in small paraffin blocks, and thin slices are shaved off the blocks for mounting on glass slides. But because only small amounts of samples are needed, there is usually a surplus of test material, along with the other tissues that were not used. Each study also yields reams of documentation, chronicling both how the study was conducted and the results; some studies produce over 30 file cabinet drawers full of such documentation.

All of this material is housed in the NTP archives. The archives, the only one of its kind in the world, functions as a huge library. Its collection is vast, including 8–10 million glass slides and over 8,000 frozen specimens, along with uncounted numbers of paraffin blocks, wet specimens, and paper and microfiche documents.

Steve McCaw, Image Associates

A wealth of information. The documentation for a single study can fill as many as 30 file drawers.

The paraffin blocks and histologic slides are kept in the archives indefinitely. Other tissue samples, usually larger pieces of tissue such as whole organs or animal carcasses, include specimens from both treated and untreated control animals. The length of time that materials are kept depends upon their method of storage and relevance to the NTP. Wet samples, fixed in formaldehyde, are kept for up to 10 years, which is near their usual practical shelf life. But frozen tissues have only been kept in the archives since 1994, and their usable shelf life has not yet been determined. Finally, the archives keeps paper documentation for 10 years before it is reproduced as microfiche copies, which are kept indefinitely.

. . . And How Is It Used?

Archival materials are used to review the results of particular studies in order to verify their adequacy, as well as to conduct more in-depth research on specimens that were first used prior to the development of new techniques. Robert Maronpot, project officer for the archives and chief of the laboratory of experimental pathology at the NIEHS, explains, "These revisitations of previously completed NTP bioassays may involve reassessment and grading of pathologic lesions, considerations of alternative ways to classify lesions, application of special staining techniques to assess the role of enhanced cell proliferation in induction of cancer, or using archival material in the application of modern molecular biology techniques to tease out the mechanistic basis of the observed response."

The science of molecular biology has blossomed since the archives came into being. The use of computers and more advanced equipment in scientific studies also makes it possible to take research to new levels. The NTP archives is invaluable in allowing scientists to recreate and expand studies that were first conducted before the technology was available to answer, or even ask, certain questions, such as how carcinogenesis occurs on a molecular level. Mel Hamlin, director of the archives, explains another virtue of the archives: "Normally, a pathologist might encounter a particular rare lesion maybe once or twice during the course of a career. But with the [samples from] more than 300,000 rats and 300,000 mice here, you're able to look at maybe 20 examples of that lesion." This ability to compare larger numbers of cancerous tissues enables scientists to more accurately and precisely characterize different diseases and disease processes.

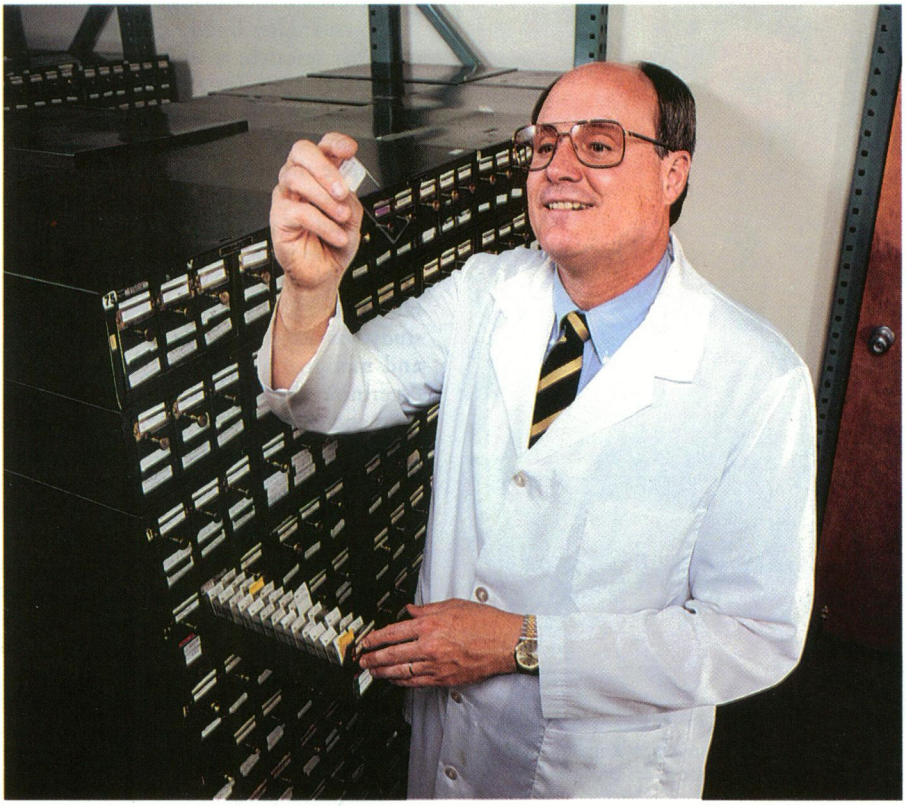
Over 28,000 researchers have visited the archives since it opened. Almost half of these came during the first five years of the archives' operation, during a time in which the NTP reviewed a large number of studies to ensure retrospectively that they were adequately conducted to assess the safety of the chemicals tested and thus, the products and manufacturing processes in which such chemicals were used. Today, most users are NTP staff members seeking to ensure the validity of current studies. But authorized outside users are also welcome. Industries may wish to verify or duplicate scientific studies pertaining to their products. Government agencies may need to confirm particular findings before setting standards, as when the FDA recently examined and reproduced NTP studies that had been conducted on sodium fluoride prior to setting new standards for that substance. And academicians may take advantage of the archives in order to further their own research.

Archival materials are usually used at the archives itself. While one purpose of the archives is to preserve its massive body of research materials, the advancement of science remains the first priority. Each year, approximately five applicants submit proposals to the archives for scientific studies that will eliminate the remainder of some sample in the collection.

Layout of the Archives

The NIEHS contracts with Experimental Pathology Laboratories of Herndon, Virginina, to manage the archives. The facility is maintained under strict security to ensure the integrity of the archives. Within the facility there are two individual workrooms, two suites of workrooms with central conference rooms, and two rooms specially designed for working with fixed whole specimens. There is also a photography lab and a dark room. The archives maintains a database containing over 30,000 photographic images that came from NTP studies. These images are used as illustrations for publications or as slides for use in oral presentations.

The archival materials are stored in seven rooms, each specially designed with two firewalls and separate sprinkler, fire abatement, and HVAC systems. Six of the rooms contain second stories, one of which was specially designed to hold the weight



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Time in a drawer? Archives director Mel Hamlin reviews one of the several million slides that are housed in the archives, ready for use in future research.

equivalent of 40 sedans. One of these two-story rooms holds the collection of paraffin blocks, one holds the collection of glass slides, and two hold the paper documentation from all the studies. The three smaller rooms house the microfiche collection, three freezers where frozen tissue samples are stored at approximately -80°C, and a "miscellaneous" room, where NIEHS scientists can store soon-to-be archived materials—the research materials associated with recent projects.

The facility has a staff of 15 dedicated employees. Hamlin, a veterinary pathologist, serves not only as the archives director but also as an on-site subject matter expert who assists researchers who are working at the archives. He is assisted by archives pathologist John Peckham, also a veterinary pathologist, and Mary Ellen Sutphin, the archives supervisor. As for the remaining 12 technical staff, they have more specimens on their hands than spare time.

As studies are completed, their attendant materials are shipped to the archives. Each specimen that arrives is closely inspected to ensure it is properly identified, labeled, and packaged for storage. For example, all wet samples are double-bagged in heat-sealed plastic envelopes. The inner and outer bags are both labeled with detailed information as to what's inside and with which study it belongs. Each item is then logged into the facility's computer system and assigned a unique identification number. There are slides, blocks, wet tissues, and data to be stored and cabinets to be labeled. And once a study's records have been converted to microfiche, each sheet of film must be examined to ensure it contains all the necessary pages of documentation before the paper copy is destroyed.

A History Lesson

All new materials that are developed by industry are required to be rigorously tested by the EPA or the FDA to ensure they are safe for consumer use. But what about all the chemicals, compounds, and materials that were introduced into consumer society before the advent of this more rigorous approach to testing? These products are the focus of the NTP's scrutiny. An NTP study is initiated when a deficit of information is identified for a potential toxin. Regulatory agencies, consumer groups, individual scientists—basically anyone who can stake a valid claim—can recommend agents for investigation by the NTP. Each year, a review board examines the nominations and ranks them in order of potential hazard and scope of effect in terms of how many people are potentially exposed to the agent in question. Substances that are not approved for investigation are rolled over to the next year for reconsideration.

Each year, the NTP begins approximately 12 new two-year bioassays designed to study the effects, sources, causes, and diagnoses of cancer and other endpoints at a cost of approximately $4 million each. In addition, 12–15 three-month toxicity studies are also undertaken. The NTP was originally established in the late 1970s by combining resources from the FDA, the EPA, the Occupational Safety and Health Association, and the National Cancer Institute, under the auspices of the National Cancer Act of 1971. The NTP contracts with several testing laboratories around the country to perform the actual tests of the materials, and enforces rigid guidelines on how the studies are to be performed.

In addition to the laboratory reports, the NTP publishes an independent technical report for each NTP study. The technical report presents the NTP's interpretation of the study's findings. Prior to publication of the technical report, a retrospective audit is conducted by an independent quality assurance contractor.

Practical Applications

The chief use of archival materials is for assessment of current studies and retrospective reviews of past studies. Reviews might serve to confirm the study's original results or suggest expansion of the study to include the use of more sophisticated experimentation methods. For example, in research that was published in both the May 1993 issue of *Carcinogenesis* and the December 1994 issue of *Cancer Research*, archival tissue samples were used to perform a retrospective analysis of molecular alterations in methylene chloride-induced lung and liver tumors. Since the time the original study was performed, the carcinogenic effects of methylene chloride had become the subject of closer scrutiny due to recognition of the prevalence of the chemical in industry, food preparation, and agriculture. The archived samples were, thus, tested with state-of-the-art technology in which DNA fragments were isolated from the tumors and screened for H-*ras* codon 61 mutations. The new results were used to help interpret the mechanism of tumor induction by methylene chloride, and it was concluded that the compound probably promoted spontaneous background tumors normally observed in the mice.

Steve McCaw, Image Associates

Treasure trove. Archives supervisor Mary Ellen Sutphin and senior archives specialist Kenneth Connolly use one of several mini-laboratories that are available for investigators to work on-site with archival materials.

Studies may also be reviewed as scientific advances alert researchers to new factors and events that should be examined. For example, one retrospective study used archived tissues from an earlier study in which *o*-nitrotoluene, a chemical used in pesticides, pharmaceuticals, and the manufacture of dyes, was determined to induce precancerous liver lesions. In the contemporary study, published in the 16 August 1995 issue of *Cancer Letters*, NIEHS investigators sought to further establish whether *o*-nitrotoluene-induced lesions would increase or decrease in size following abatement of exposure to the chemical. The tissues were stained for placental glutathione *S*-transferase and were monitored for periods of 13 and 26 weeks. The findings demonstrated that, once exposed to *o*-nitrotoluene, the chemical can be expected to induce hepatocarcinogenesis even if the exposure is stopped.

Some studies build upon the findings of earlier research, as in a study published in the July 1995 issue of *Carcinogenesis* in which investigators used archival tissues containing ozone-induced lung tumors to determine the relationship between ozone and mutations in the mouse K-*ras* gene. NTP studies had established that long-term ozone inhalation increases the incidence of lung tumors in B6C3F1 mice, but not in F344/N rats. The *Carcinogenesis* study was designed to evaluate both benign and malignant lung tumors in mice to determine the frequency of K-*ras* mutations and whether such mutations could be proven to be associated with ozone inhalation. The study's findings indicated that ozone does indeed cause mutation of the K-*ras* gene in B6C3F1 mice.

The sheer mass of data on rodent pathology that has been accumulated in the archives has afforded NTP scientists the ability to publish full reference atlases on the subject. *Pathology of the Fischer Rat*, published in 1990, is considered one of the most authoritative texts in its area. A second reference text, *Pathology of the B6C3F1 Mouse*, is currently in production. Material reviews have also been used for contributions to other books on rat and mouse pathology, as well as in the preparation of conference programs for NTP meetings. The NTP archives has much to offer today's researchers, including the promise implicit in the act of preserving the archives that any question, once asked, can someday be answered.

Susan M. Booker